IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**09/932,521**
Applicants	:	**Hans Herweijer**
Filed	:	**08/17/2001**
Art Unit	:	**1632**
Examiner	:	**Woitach, Joseph T.**
Docket No.	:	**Mirus.023.01**
For	:	**Nucleic Acid Expression From Linear Nucleic Acids**

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Mark K Johnson

APPELLANT'S BRIEF

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1. Real party in interest:

The real parties in interest are: Hans Herweijer and Christine I. Wooddell and, by assignment, Mirus Corporation, which has changed its name to Mirus Bio Corporation under the laws of the State of Delaware and is located at 505 South Rosa Road, Madison, WI 53719.

2. Related appeals and interferences:

There are no interferences known to appellant, the appellant's legal representative, or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

3. Status of Claims:

Claims 1-15 and 17 have been rejected and are hereby appealed.

Claims 16 and 18-20 have been canceled.

4. Status of amendments:

No Amendments have been filed subsequent to the rejection.

5. Summary of claimed subject matter:

The claimed subject matter is a process for improving expression of a transgene *in vivo*. It has frequently been found that the persistence of expression of a heterologous gene delivered non-virally to a cell *in vivo* is relatively short. The standard practice in the art is to isolate DNA from bacteria in the form of closed circular supercoiled plasmids. The plasmid DNA is then directly delivered to the cell in vivo. Expression from such plasmid DNA can be substantially reduced in as little as one day. Applicants have discovered that, by linearizing the plasmid prior to delivery to cells, expression of the delivered gene persists for a substantially longer period of time. The preferred methods for linearization of the plasmid, restriction enzyme digestion and polymerase chain reaction, are well known in the art.

Covalently closed circular plasmid DNA was thought to be more stable *in vivo*, since exonucleases, which digest DNA from terminal ends, would not have access to a circular plasmid. However, Applicants have shown detectable expression from a transgene delivered as a linear DNA for as long as 182 days. The same transgene, when delivered as a closed circular supercoiled plasmid, was undetectable after 28 days (specification page 19, line 18). Increased expression from linear DNA presents at least two important improvements over the standard process of circular plasmid gene delivery *in vivo*. The first advantage is the obvious benefit of improved expression over a longer period of time. Second, because linear DNA is used, bacterial sequences, which must be present in plasmid DNA for its production and has been shown to be immunogenic, can be eliminated. Removal of such sequences reduces toxicity and immune-related suppression of transgene expression.

Applicants have claimed a process for *in vivo* expression of longer than seven days of a non-viral, linear DNA nucleic acid sequence from a delivered expression cassette. The expression cassette comprises the nucleic acid sequence operably linked to a promoter (specification, page 12, lines 32-34 and page 13, lines 1-11). Then, a non-viral, linearized plasmid DNA vector is formed comprising the expression cassette (specification, page 20, lines 8-10 and pages 21-22, examples 1 and 2). Subsequently, delivering the non-viral, linearized plasmid DNA vector to a hepatocyte in a mammal, wherein providing the expression cassette on the non-viral, linearized plasmid DNA

vector results in increased expression in the hepatocyte after seven days defined by at least 20% more gene product than is expressed from a supercoiled plasmid from which the linearized plasmid is derived (specification, pages 22 and 23, examples 3, 4 and 5).

Human factor IX (ng/ml) was measured in the plasma averaged from mice as shown in the following table (specification page 23, line 7).

	Human factor IX expression level in mouse plasma (in ng/ml) at various days after pDNA injection			
Injected DNA	**Day 1**	**Day 7**	**Day 28**	**Day 62**
Supercoiled plasmid	55,620	9	< 1	<1
Blunt-linearized plasmid	48,169	119	137	385

6. Grounds of rejection to be reviewed on appeal:

Whether claims 1-15 and 17 are unpatentable under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed has possession of the claims invention.

Whether claims 1-15 and 17 are unpatentable under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

7. Argument:

Rejection of claims 1-15 and 17 under 35 U.S.C. 112, first paragraph: The Action states, on page 3, that there is a lack of specific teaching for the invention as claimed and that there is not adequate guidance to practicing the breadth of the claims. The Action states

> "*... the literal support for the terminology is acknowledged, however the specification contemplates the use of a variety of promoter types including conditional promoters which would not necessarily be active at injection or at seven days. The basis of maintaining the rejection is that the claims are drawn to specific embodiments set forth in the specification which were not specifically contemplated together.*"

The Action goes on to state that expression levels will depend on the promoter, means of delivery, and stability of the gene product itself.

Applicants do not dispute that these factors will affect the overall level of gene expression. However, choosing an appropriate promoter is a matter of experience and preference that is well known by one having skill in the art as well as choosing the means of delivery. The stability of the gene product is provided in claim 1 as "*increased expression in the hepatocyte after seven days defined by at least 20% more gene product than is expressed from a supercoiled plasmid from which the linearized plasmid is derived.*"

Applicants have shown that expression of a DNA sequence persists for a longer time and at higher levels if the expression cassette is delivered to the cell *in vivo* as a linear DNA rather than the same sequence in the known delivery vehicle, a covalently closed circular plasmid.

Rejection of claims under 35 U.S.C. 112, first paragraph: The Action states, on the bottom of page 3 to the top of page 4 that the specification must teach those of skill in the art how to make and how to use the invention as broadly claimed. Applicants believe this requirement has been met. Applicants have taught that the injection of naked plasmid DNA (pDNA) into liver or tail vein vessels leads to high levels of

foreign gene expression (specification page 4, line 1). Efficient methods for delivering plasmid DNA is described in the specification beginning on page 4, line 25 in the section entitled Delivery of Nucleic Acids (references provided in the evidence appendix).

One with skill in the art must provide the gene to be delivered in the form of a linear DNA rather than an art-standard circular plasmid. The means for forming a linear DNA are described in the specification on page 7, beginning on line 13 in the section entitled Linear DNA. Other methods are well established in the art.

Therefore, Applicants believe that they have provided sufficient support in the specification to show that the new matter rejection is groundless and to enable one skilled in the art to make and/or use the invention.

Rejection of the claims 1-15 and 17 under 35 U.S.C. 112, second paragraph: The Action states that the claims are indefinite because there is no embodiment that requires the expression cassette to be active in the liver.

The claims recite delivering the linearized plasmid DNA vector to a hepatocyte, a liver cell, in a mammal. It is well known by one skilled in the art that expression in a hepatocyte requires a promoter that is active in the liver. Such promoters are readily recognized and determined. On page 12, line 32 of the specification in the section entitled Expression Cassette, Applicants state that "A DNA expression cassette typically includes a promoter (allowing transcription initiation)." On page 20, line one, the statement "Expression cassettes that contain sequences to be transcribed in mammalian cells require a promoter of mammalian or viral origin" indicates that one having skill in the art would have knowledge of such promoters.

It is well known that liver active transcription factor/promoters in the art include: viral promoters such as CMV, RSV, hepatitis B, and SV40 promoters, and liver promoters/factors such as albumin promoter, glucokinase promoter, CCAAT/enhancer binding protein, connexin 32 gene B2 element, D-site binding protein, hepatic leukemia factor, hepatic nuclear factor, insulin response element, metal response element, methylated DNA-binding protein site, peroxisome

proliferator response element, promoter linked coupling element, xenobiotic response element, alpha-fetoprotein promoter, alpha1-antitrypsin promoter.

The Action also states that "*there is no embodiment within the claims that requires ... functional properties to assess expression levels at a later time*." Applicants are claiming a process for expression of DNA and do not believe that it is necessary for them to include "*functional properties*" in the context of the claims or why expression assessment is expected to be recited in the claims. Assessment of gene expression is routinely practiced by those skilled in the art. Methods of assessing gene expression practiced in the art include: Northen Blot analysis, ELISA, reverse transcriptase PCR, Western blot analysis, and enzyme assays.

8. Claims Appendix:

1. (previously presented) A process for *in vivo* expression of longer than seven days of a non-viral, linear DNA nucleic acid sequence from a delivered expression cassette, comprising:

   a) providing the expression cassette comprising the nucleic acid sequence operably linked to a promoter;

   b) forming a non-viral, linearized plasmid DNA vector comprising the expression cassette; and,

   c) delivering the non-viral, linearized plasmid DNA vector to a hepatocyte in a mammal, wherein providing the expression cassette on the non-viral, linearized plasmid DNA vector results in increased expression in the hepatocyte after seven days defined by at least 20% more gene product than is expressed from a supercoiled plasmid from which the linearized plasmid is derived.

2. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector contains blunt ends.

3. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector contains sticky ends.

4. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector contains a blunt end and a sticky end.

5. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector is generated by restriction enzyme digestion.

6. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector is generated by polymerase chain reaction.

7. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector contains an expression cassette isolated from a plasmid backbone.

8. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector contains an expression cassette which is flanked by sequence derived from inner Tn5 transposase recognition elements.

9. (previously presented) The process of claim 8, wherein the non-viral, linear DNA vector ends are blunt.

10. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector contains an expression cassette which is flanked by sequence derived from outer Tn5 transposase recognition elements.

11. (previously presented) The process of claim 10, wherein the non-viral, linear DNA vector ends are blunt.

12. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector contains an expression cassette which is flanked by chimeric ends derived from Tn5 transposase recognition elements.

13. (previously presented) The process of claim 12, wherein the non-viral, linear DNA vector ends are blunt.

14. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector is delivered to cells intravascularly.

15. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector are delivered intravascularly using pressure.

16. (canceled)

17. (previously presented) The process of claim 1, wherein the non-viral, linear DNA vector is delivered by direct interstitial injection.

18. (canceled)

19. (canceled)

20. (canceled)

9. Evidence appendix:

Copies of the references cited in the specification beginning on page 4, line 25 from the section entitled Delivery of Nucleic Acids can be found in this appendix. The cited references are:

G. Zhang, V. Budker and J.A. Wolff. Human Gene Therapy 10:1735-1737, 1999.

V. Budker, G. Zhang, S. Knechtle and J.A. Wolff. Gene Ther. 3:593-598, 1996.

G. Zhang, D. Vargo, V. Budker, N. Armstrong, S. Knechtle and J.A. Wolff. Hum.

10. Related proceedings appendix: None.